Electronic Supplementary Material (ESI) for Lab on a Chip
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A microfluidic cell culture array with various oxygen tensions

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Supporting Information

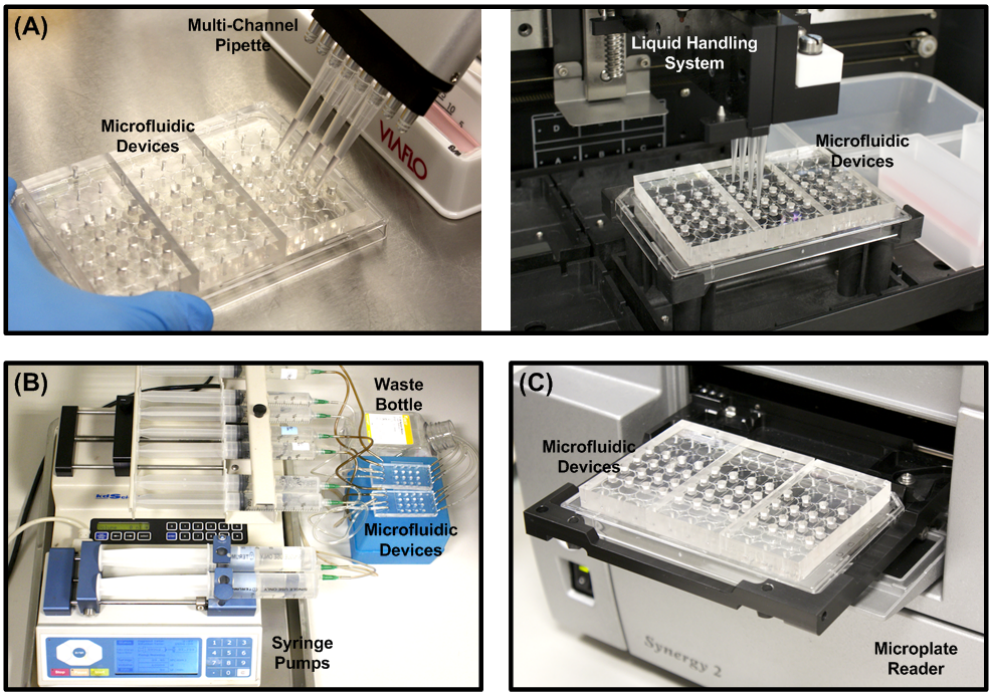

Fig. S1 Photos of operation of the device. (A) The cell culture wells on the device are arranged with the same dimensions as conventional 96-well microplates; therefore, conventional multi-channel pipettes and liquid handing systems (Precision Microplate Pipetting System, BioTek Instruments, Inc., Winooski, VT) can be exploited to seed cells and perform assays on the designed device. The photos show that three microfluidic devices (each has 4x4 wells) are placed onto a 96-well microplate cover for simultaneous operation (cell seeding or viability assay). (B) Photo of the experimental setup with two microfluidic cell culture arrays capable of simultaneously generating 8 different oxygen tensions. One ten-syringe syringe pump is utilized to inject pyrogallol solutions with different concentrations to generate various oxygen tensions inside the cell culture wells. One double-syringe syringe pump is used for NaOH injection. The syringes with blunt needles and tubings are connected to the devices only during the drug testing on the cell culture. The entire setup occupies around 1/3 space of a cell incubator.[S1] (C) Photo of three microfluidic devices loaded into a plate reader (Synergy 2, BioTek Instruments, Inc.) for assay readings after the cell culture experiments.

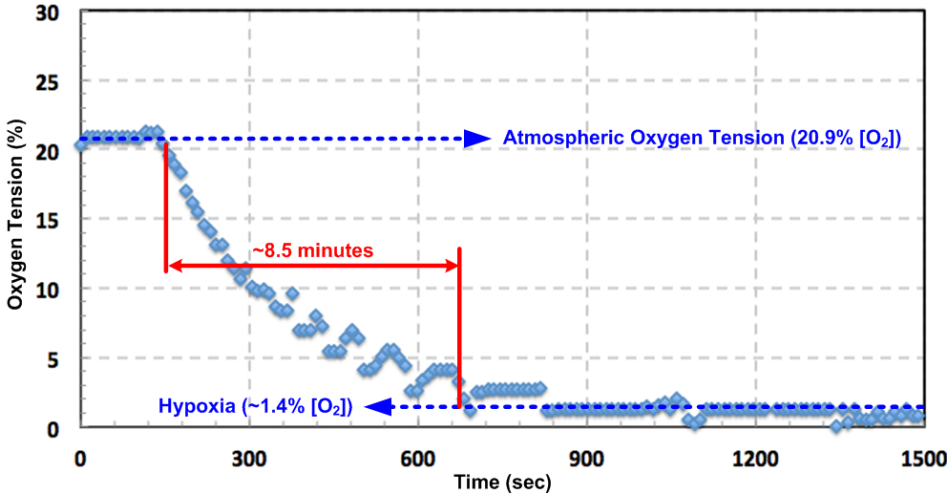

Fig. S2 Experimentally measured temporal response of the oxygen tension within the cell culture well. The result shows that the oxygen tension for cell culture is lowered and reached steady state within approximately 8.5 minutes after introducing the chemical reactants for oxygen scavenging into the fluidic channels on the bottom layer. The slightly longer time to reach the steady state, comparing to the simulated one (approximately 5 minutes), may result from additional oxygen dissolved in the PDMS that are not considered in the numerical simulation model.

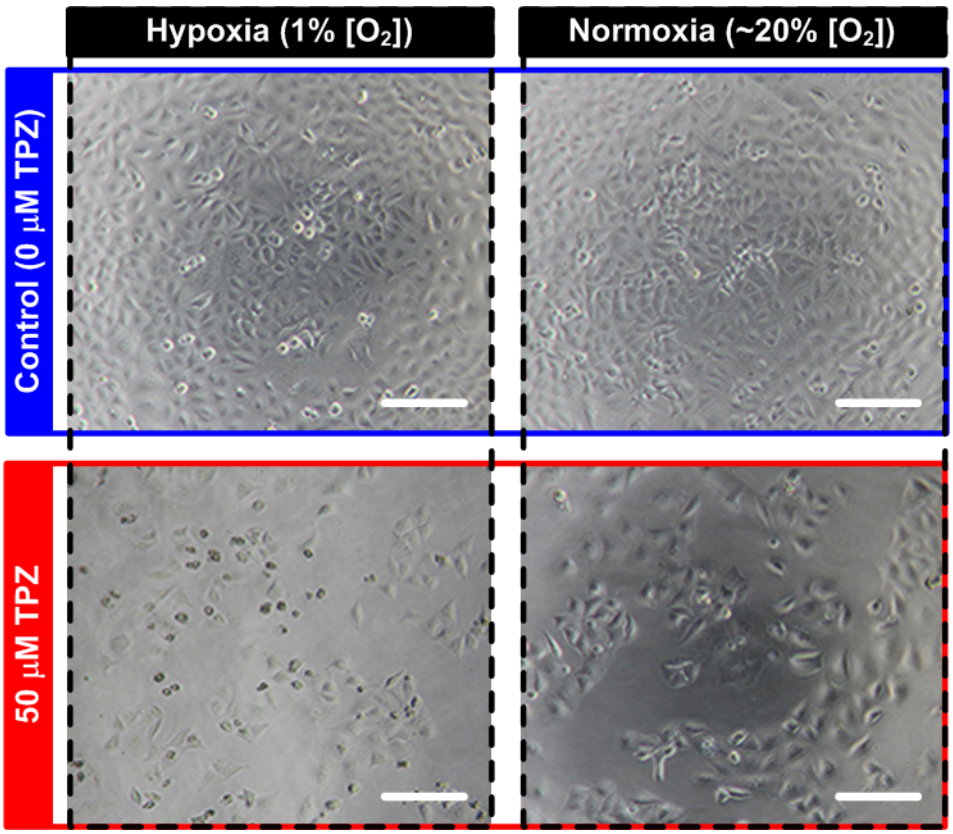

Fig. S3 Bright field phase images of A549 cells cultured in a conventional 96-well plate (167008, Nunclon DELTA Surface, Nunc, Roskilde, Denmark) after 48 hours with 0 and 50 μM TPZ under hypoxia (1% [O_2]) controlled by a cell incubator (Heracell 240i, Thermo Scientific, Waltham, MA) and normoxia (~20% [O_2]) conditions. Scale bar is 100 μm.

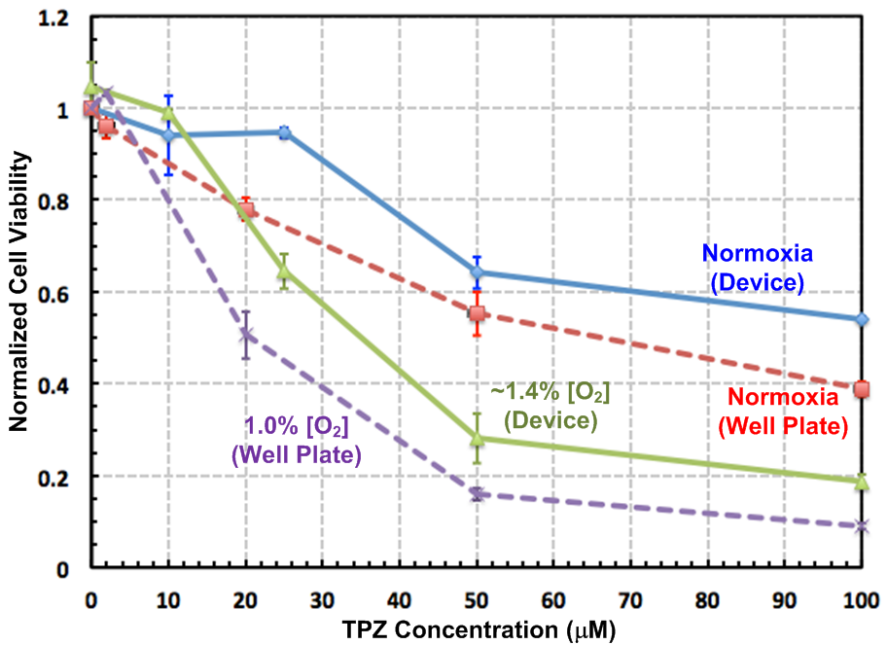

Fig. S4 Comparison of cell viabilities under various drug treatments in normoxia and hypoxia conditions using the developed cell culture arrays and conventional 96-well plates (167008, Nunclon DELTA Surface, Nunc) with an oxygen tension-controlled cell incubator (Heracell 240i, Thermo Scientific). In the well plate experiments, approximate 5000 A549 cells were seeded into each well. The cells with 80 μl medium were incubated overnight in the cell incubator to ensure the cell attachment onto the wells before the drug treatments, and 20 μl drug solutions with various TPZ concentrations were than added into the wells. The cells were incubated with drug-contained medium for 48 hours. After the testing, the cell viabilities were estimated using the fluorescence-based cell viability assay, and read by the plate reader. The drug response curves obtained using the developed devices and the well plates are similar for both normoxia and hypoxia conditions. The slightly low viabilities in the well plate experiences may result from the low gas oxygen permeability of polystyrene, which is used to make well plates. Therefore, the real oxygen tensions that cells experienced would be lower than that set in the incubator.[S2] As a result, the drug shows more hypoxia-activated cytotoxicity of TPZ to A549 cells comparing to that obtained using the developed device. Data are expressed as the mean ± s.e.m. (n = 4 for the device experiments; n = 8 for the well plate experiments).

Reference

S1 Y.-A. Chen, A. D. King, H.-C. Shih, C.-C. Peng, C.-Y. Wu, W.-H. Liao and Y.-C. Tung, *Lab Chip*, 2011, **11**, 3626.

S2 A. V. Zhdanov, V. I. Ogurtsov, C. T. Taylor and D. B. Papkovsky, *Integr. Biol.*, 2010, **2**, 443.